American Journal of Hematology 58:299–305 (1998)

Development of Iron Chelators to Treat Iron Overload Disease and Their Use as Experimental Tools to Probe Intracellular Iron Metabolism

D.R. Richardson[1]* and P. Ponka[2,3]

[1]Department of Medicine, Royal Brisbane Hospital, Herston, Brisbane, Queensland, Australia
[2]Lady Davis Institute for Medical Research of the Sir Mortimer B. Davis Jewish General Hospital, Montréal, Québec, Canada
[3]Departments of Physiology and Medicine, McGill University, Montréal, Québec, Canada

The development of an orally effective iron (Fe) chelator for the treatment of Fe overload diseases such as β-thalassemia has been a difficult challenge. Even though the drug in current clinical use, desferrioxamine (DFO), is efficient and remarkably free of toxicity, it suffers from not being orally effective and requiring long subcutaneous infusion to mobilize sufficient quantities of Fe. In addition, DFO is very expensive, which precludes it from treating most of the world's thalassemic population. Therefore, the development of an economical and orally effective Fe chelator is of great importance. Despite the screening of a wide range of structurally diverse ligands from both natural and synthetic sources, few compounds have been promising enough to proceed to clinical trials. In the current review, the properties of an ideal chelator are discussed, followed by a description of the most successful ligands that have been identified. Apart from the use of Fe chelators as therapeutic agents, some of these compounds have also been useful as experimental probes to investigate cellular Fe metabolism. We describe here the most important of these studies. Am. J. Hematol. 58:299–305, 1998. © 1998 Wiley-Liss, Inc.

Key words: iron chelation therapy; desferrioxamine; iron chelators; iron overload disease

INTRODUCTION

The development of selective iron (Fe) chelators for the treatment of Fe overload diseases such as β-thalassemia is an area of much current interest [1–3]. Apart from being used to treat Fe overload, these compounds may also be useful therapeutic agents against a number of conditions [4], including some cancers [5–9], malaria [10–12], and free radical-mediated injury [13–17]. At present, the only Fe chelator in widespread clinical use is the tris-hydroxamate desferrioxamine (DFO). However, this drug suffers a number of important disadvantages such as its high cost, its requirement for long subcutaneous infusion (12–24 hr/day, 5–6 times per week), and its poor absorption from the gut. In addition, there is evidence that administration of DFO can result in an allergic response due to activation of mast cells [18–20]. This latter point is a major issue relative to the discomfort patients experience at the injection site. All these deficiencies of DFO are significant, as β-thalassemia occurs at its greatest frequency in developing countries where expensive drug regimes cannot be implemented. Therefore, the development of an economical, effective, and orally absorbed Fe chelator is critical in terms of treating most of the world's thalassemic population [21].

In recent years we have witnessed the development of a new class of Fe chelators known as the α-ketohydroxypyridones that were specifically developed to replace desferrioxamine (DFO). The most promising of this class of ligands is 1,2-dimethyl-3-hydroxypyrid-4-

Contract grant sponsor: Medical Research Council of Canada; Contract grant sponsor: National Cancer Institute of Canada; Contract grant sponsor: National Health and Medical Research Council of Australia; Contract grant number: 97060; Contract grant sponsor: Department of Medicine, University of Queensland.

*Correspondence to: Dr. D.R. Richardson, Department of Medicine, Clinical Sciences Building Floor C, Royal Brisbane Hospital, Herston, Brisbane, Queensland 4029, Australia. E-mail: D.Richardson@medicine.herston.uq.edu.au

Received for publication 26 March 1998; Accepted 8 April 1998

one (also known as L1, DMHP, CP20 deferiprone), which is orally effective and shows high Fe chelation efficacy [2,22,23]. Unfortunately, recent results have demonstrated that there are limitations with deferiprone therapy [3,24–28], and there is some controversy regarding its efficacy in reducing hepatic Fe levels [3,29]. These latter data suggest that the quest for an alternative Fe chelator to DFO should not be limited to deferiprone or its analogues. In fact, several other ligands have been identified that have high Fe chelation activity, including pyridoxal isonicotinoyl hydrazone (PIH) and the phenolic EDTA analogues, both of which deserve further investigation.

In the current review, we will examine the properties that are required for a clinically useful Fe chelating agent and then discuss the most efficient compounds that have reached clinical trials. Many ligands that were designed to treat Fe overload have also been useful experimental probes to investigate cellular Fe metabolism, and in the last part of the review we will describe some of the studies that have used this strategy.

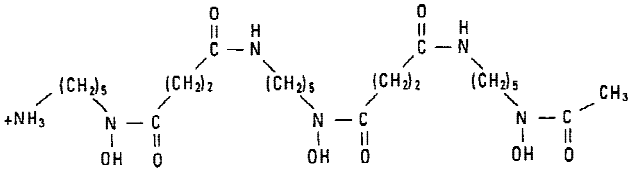
DEVELOPMENT OF CHELATORS TO TREAT IRON OVERLOAD DISEASE

The search for clinically useful iron (Fe) chelators has been a protracted and difficult exercise that still remains a challenging goal. Before discussing the range of compounds that have been examined, it is necessary to describe the properties of an ideal Fe chelator, which have been defined by Chaberek and Martell, [30]. First, the ligand must be biospecific, that is, it must have a high affinity for Fe in vivo compared to hemosiderin, ferritin, and transferrin, but low when compared to hemoglobin, myoglobin, and the cytochromes. In addition, the chelator should have low affinity for all other physiologically important cations other than Fe(II) or Fe(III). Second, the compound must be bioavailable, preferably suitable for oral administration, absorbable from the gut, and transportable at an effective concentration in the bloodstream. An ideal chelator should also bind Fe rapidly in competition with other natural Fe-binding proteins, notably transferrin. Third, the ligand should be stable to hydrolytic and enzymatic degradation prior to and after absorption. Fourth, the chelator must be biocompatible, having minimum side effects both acute and cumulative. The drug should form an Fe complex that is lipophilic enough to diffuse out of cells but should not be too lipophilic in order to prevent accumulation within cell membranes or adipose tissue. Fifth, the ideal chelator should cause an as large as possible excretion of Fe per unit weight of drug administered, both for economy and ease of application. Finally, the compound should involve reasonably inexpensive starting materials and relatively few synthetic steps.

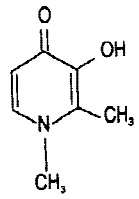
Desferrioxamine and Other Siderophores

Initial investigations to obtain an ''ideal Fe chelator'' were focused on microbial Fe transport molecules known as siderophores. Siderophores are defined as low molecular weight Fe transport agents elaborated by aerobic and facultative anaerobic microorganisms under low Fe stress [for review see 31]. Microbes primarily use two types of chemical groups for Fe chelation, namely hydroxamic acids (-N(OH)-C = O) and catechols (two adjacent hydroxyl groups on a benzene ring). However, this classification is not precise, since desferrithiocin and the siderophores, which are derived from citric acid, contain in addition, carboxylate, hydroxyl, and nitrogen groups that can bind Fe. Desferrioxamine (DFO), which is also known as Desferal® (Ciba-Geigy, Basle, Switzerland), is the only siderophore that has been used clinically with success [for reviews see 3,32]. This drug is a naturally occurring sideramine synthesized by *Streptomyces pilosus*, and is a tris hydroxamate Fe chelator (Fig. 1) that binds Fe(III) very tightly. In fact, its binding constant (log β) for Fe(III) is similar to that of Tf(10^{29}) but is appreciably lower for other biologically important elements (from 10^{14} for copper(II) to 10^{2} for calcium(II) [33]. Desferrioxamine is highly selective, and can only slowly remove Fe from ferritin and hemosiderin [33], but not from hemoglobin, myoglobin, cytochromes, oxidases, catalase, or peroxidase [34]. Unfortunately, while DFO can be absorbed from the gut, the urinary Fe excretion is only 1–10% of the amount that is mobilized after a subcutaneous infusion of the same dose, precluding this route of administration [35]. Desferrioxamine is cleared rapidly from the plasma, and has a half-life of 5–10 min [36], which necessitates long subcutaneous infusion. Propper and associates [37] demonstrated the superiority of continuous infusion of DFO as opposed to bolus injection in enhancing urinary Fe excretion. For example, if a 750 mg standard dose of DFO is administered as a continuous 24 h infusion, it promotes the excretion of 3–4 times the amount of Fe as a single intramuscular injection [37,38]. The amount of Fe excreted by DFO is variable and is probably dependent upon a number of factors, including the patient's age, the total body iron load, and the number of blood transfusions [39,40]. In fact, the dose of DFO to be given to each patient is best determined by performing a dose-response curve [38,40,41].

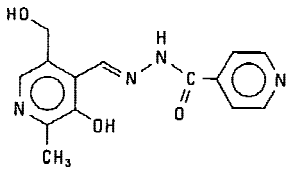
The clinical success of DFO suggested that other siderophores may be identified that have better properties including the ability to be absorbed from the gut. With this aim in mind, a large variety of siderophores have been isolated from a range of microorganisms. Despite examination of several of the most efficient of these chelators, including rhodotorulic acid and desferrithiocin, none have shown more promise than DFO. In fact,



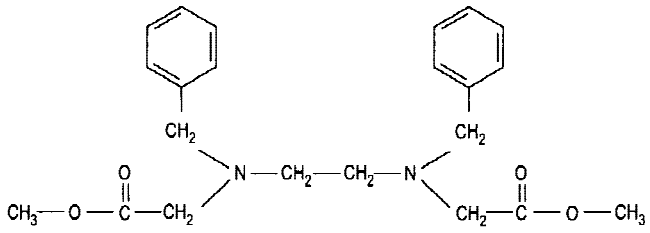
desferrioxamine (DFO)



1,2-dimethyl-3-hydroxypyrid-4-one (L1)



pyridoxal isonicotinoyl hydrazone (PIH)



Dimethylester of N,N′-bis(o-hydroxybenzyl) ethylenediamine diacetic acid (dm HBED).

Fig. 1. Structures of effective iron chelators: desferrioxamine (DFO), 1,2-dimethyl-3-hydroxypyrid-4-one (also known as L1, deferiprone, CP20 or DMHP), pyridoxal isonicotinoyl hydrazone (PIH), and the dimethylester of N,N′-bis(o-hydroxybenzyl) ethylenediamine diacetic acid (dmHBED).

rhodotorulic acid is not orally effective and was very painful after injection [42], while the Fe complex of desferrithiocin was toxic [43]. It is also relevant to note that siderophores suffer a number of disadvantages. First, they may promote infection by donating Fe to microbes ranging from pathogens to common enteric bacteria. Second, even under low Fe stress siderophores are not always produced in high yields. Third, since their chemical structures may be complex, their chemical synthesis may be too expensive. While these concerns are important, it should be pointed out that not all pathogens can cross-utilize siderophores, and modern culture techniques and genetic engineering can overcome the obstacle of low yields. However, to overcome the potential problems of siderophores, extensive effort has been invested to develop synthetic chelators.

To date, the most effective synthetic ligands fall into three main classes, namely the hydroxypyridones, pyridoxal isonicotinoyl hydrazone (PIH) and its analogues, and the phenolic EDTA analogues such as N,N′-bis(2-hydroxybenzyl) ethlenediamine-N,N′-diacetic acid (HBED). These compounds have all reached clinical trials over the last 10 years, the α-ketohydroxypyridones (α-KHPs) being the most well studied.

α-Ketohydroxypyridones

The α-KHPs are small bidentate chelators (Fig. 1) that diffuse quickly across cell membranes to chelate intracellular Fe pools [44,45]. In contrast to many other Fe chelators, these compounds efficiently remove Fe from the serum Fe transport protein transferrin (Tf) both in vitro [46] and in vivo [47]. This latter property is probably quite significant, since although Tf represents only a very small fraction of total body Fe, it is the main extracellular Fe transport protein that is involved in Fe exchange between tissues. Like most Fe chelators (including DFO and the PIH analogues), the α-KHPs can inhibit ribonucleotide reductase activity by binding intracellular Fe pools and preventing DNA synthesis [48]. Some of these chelators can also remove Fe from isolated ferritin [49], the protein that is involved in cellular Fe storage [50]. The ability of these chelators to bind Fe from a variety of major Fe-containing proteins is probably partially responsible for their high Fe chelation activity in vivo.

As described previously, the most well-studied compound of this series of chelators is deferiprone. Since 1987 approximately 1,000 patients in 16 countries have taken this drug during clinical trials or in situations where DFO cannot be used. While licensing of deferiprone has been deferred by the United States Food and Drug Administration (FDA), the drug has been licensed for sale in India [3]. A number of studies have shown that deferiprone can reduce tissue Fe stores in Fe-loaded patients [23,51,52]. However, recent results from a long-term Canadian trial in thalassemic patients have contradicted these findings [3,26,27]. Considering this latter investigation, in patients that had completed 2 years of study, hepatic iron concentrations were 50% over baseline levels in patients treated with deferiprone [3]. These results have raised the concern that deferiprone may not provide adequate control of body Fe in a substantial proportion of patients with thalassemia major. Apart from this problem, it must also be pointed out that there are

some adverse effects associated with deferiprone therapy, the most common being arthralgias, and most serious being severe neutropenia or agranulocytosis [24,25,28,53]. While further studies with the α-KHPs should continue, the limitations associated with deferiprone suggest that other classes of compounds should not be ignored in the quest for alternatives to DFO.

Pyridoxal Isonicotinoyl Hydrazone and Its Analogues

Pyridoxal isonicotinoyl hydrazone (PIH; Fig. 1) was initially developed by Ponka and colleagues after experiments showed that it markedly increased[59] Fe release from reticulocytes loaded with non-haem ^{59}Fe [54]. Subsequent studies showed that this ligand was orally effective in vivo in experimental animals having an efficacy similar to that observed with parenteral DFO [55–59]. Importantly, the PIH group of chelators have a high affinity and selectivity for Fe and low affinity for other biologically important ions such as Ca(II) and Mg(II) [60,61]. In fact, their selectivity for Fe(III) is similar to that of DFO and much greater than that of EDTA or DTPA [60]. These chelators are tridentate and have a neutral charge at physiological pH allowing passage through cell membranes and access to intracellular Fe pools [62]. Mobilization of ^{59}Fe mediated by PIH is an active process, and in fact, the Fe complex may actually be transported out of cells by an energy-dependent carrier [63,64].

In an effort to improve the efficacy of PIH, analogues of this chelator have been synthesized, some of which show high activity at mobilizing Fe from both normal and neoplastic cells [9,65–68]. Some of these compounds, particularly those derived from salicylaldehyde and 2-hydroxy-1-naphthylaldehyde, show high Fe chelation activity and marked antiproliferative effects [67,68]. In contrast, other hydrazones derived from pyridoxal also show high Fe chelation activity but low anti-proliferative effects, and these compounds may have potential as agents to treat Fe overload [67].

Despite the high activity of PIH in both in vitro and in vivo experiments, only 1 clinical trial with this chelator has been reported [69]. In this latter study, orally administered PIH caused sufficient Fe excretion (0.12 ± 0.07 mg/kg/day) to maintain a negative Fe balance in patients with Fe-loading anaemias that were not regularly transfused [69]. In addition, in these patients, there were no signs of toxicity from treatment with the drug. However, in regularly transfused patients, a greater excretion of Fe (approximately 0.5 mg/kg/day) would be necessary to match the Fe overload derived from transfusions [69]. Although this trial provided very useful information, it is unfortunate that PIH was given as a powder in gelatin capsules [69], as PIH has low solubility in aqueous solutions and it is likely that it was poorly available for absorption. Hence, this investigation probably did not give a good indication of the efficacy of PIH at mobilizing Fe [70]. Obviously, further clinical trials with a bioavailable form of PIH or its analogues are essential in order to assess their potential as useful Fe chelators.

Phenolic EDTA Analogues

These hexadentate ligands are based on EDTA and have rather complex structures (e.g., see dmHBED, Fig. 1). A number of these compounds have been examined including N,N′-ethylene-bis(o-hydroxyphenylglycine) (EHPG), N,N′-bis(o-hydroxybenzyl)-ethylenediamine diacetic acid (HBED), and their respective dimethyl esters (dmEHPG and dmHBED) [71]. The most effective non-toxic compound of these chelators in rats is dm HBED, which is a prodrug of HBED and is not a hexacoordinate ligand by itself (Fig. 1). This latter drug is 15 times more effective in promoting Fe excretion than DFO when administered intramuscularly and 10 times more effective when given orally [72,73]. A limited clinical trial with HBED resulted in enhanced urinary and stool Fe excretion in all thalassemic patients studied [74]. Significantly, no signs of toxicity were seen after administration and it caused a negative Fe balance in 1 thalassemia intermedia patient [74]. In general, the efficacy of the chelator was less than that expected from animal studies, but future work was planned in order to increase its oral bioavailability [74]. More recent studies have demonstrated that orally administered HBED is not as effective as DFO and cannot place thalassemia major patients into a negative Fe balance [75].

IRON CHELATORS AS PROBES OF CELLULAR IRON METABOLISM

Apart from the use of Fe chelators as therapeutic agents, they have also been implemented to dissect the mechanisms involved in cellular Fe metabolism. For example, Thorstensen [76] described the use of Fe chelators in combination with other metabolic probes to determine the site of Fe release from transferrin (Tf) in reticulocytes compared to hepatocytes. Hydrophilic, membrane-impermeable Fe chelators such as bathophenanthroline disulphonate (BPS) were found to be effective at reducing Fe uptake from Tf only by hepatocytes, whereas hydrophobic, membrane-permeable ligands such as αα-dipyridyl reduced Fe uptake in both reticulocytes and hepatocytes [76]. Combined with data from experiments with other metabolic probes, these studies suggested that hepatocytes, in contrast to reticulocytes, have an additional Fe uptake mechanism that is at the cell surface or at a site that is accessible to extracellular chelators (e.g., pinosomes). These results confirmed work by other investigators using alternative methods [77,78]. Similarly, in human melanoma cells, extracellular Fe chelators such

as EDTA, DTPA, and BPS were found to be significantly (P less than 0.001) more effective at reducing ^{59}Fe uptake from high ^{59}Fe-Tf concentrations above saturation of the Tf receptor compared to low Tf concentrations [79]. In contrast, permeable chelators such as PIH, deferiprone, and αα-dipyridyl, were equally effective at both Tf concentrations. These data suggest that Fe was being released at high Tf concentrations at a site that was in contact with the extracellular medium [79]. Together with other experiments, this work indicated that melanoma cells have an additional Fe uptake mechanism that increases after saturation of the Tf receptor and is consistent with adsorptive pinocytosis [79,80].

Recent studies have used the fluorescent chelator calcein to investigate intracellular Fe metabolism [81,82]. When K562 cells were loaded with calcein and then exposed to Fe(II) salts such as ferrous ammonium sulphate, fluorescence was rapidly quenched. In contrast, when cells were permeabilised to Fe(II) using the ionophore A-23187, there was a rapid rise in fluorescence [81]. These results suggest that Fe(II) is transported rapidly into cells and remains in that form for at least some time. Similar results were also obtained when K562 cells were incubated with Fe(III)-Tf, suggesting the presence of a step to reduce Fe(III) to Fe(II) prior to transport through the membrane [81,82]. These data confirm experiments by others that suggest the presence of an Fe(II) transport mechanism in the cell membrane [83–85] and the existence of an intracellular pool of Fe in the ferrous state [86].

While chelators can provide very useful information in dissecting the Fe metabolism of cells, it should be noted that there are some problems with this strategy. For example, as described by Thorstensen and Aisen [87], ligands with appropriate reduction potentials and high affinity for Fe(II) ion can force reduction and release of Tf-bound Fe(III) that would not be seen under physiological circumstances. This caveat was particularly relevant in investigations examining the presence of a diferric Tf reductase (an oxidoreductase) on the cell membrane [88,89]. In these studies the membrane-impermeable Fe(II) chelator, bathophenanthroline disulphonate (BPS), was added to bind Fe released from Tf in the presence of cells or NADH and membranes. In the presence of BPS, there was a marked disturbance of the overall equilibrium, resulting in the formation of the Fe-BPS complex and the apparent reduction of Tf-bound Fe at the cell surface [87].

CONCLUSIONS

The identification of suitable orally effective Fe chelators for the treatment of iron overload disease still remains an unsolved problem. While deferiprone has shown rapid development, the most recent clinical trials suggest that there are limitations due to its toxic effects, and it is controversial whether long-term therapy with this drug can reduce hepatic Fe levels. These problems suggest that investigation of other α-KHPs such as compound 94 (1,2-diethyl-3-hydroxy-pyridin-4-one; [90]) may be useful. While both PIH and the phenolic EDTA analogues show high activity in experimental models, their activity has not been thoroughly investigated in clinical trials. In fact, in the case of PIH, it is likely that the only clinical trial with this compound did not accurately reflect the chelators' potential, as it was not administered in a bioavailable form. In conclusion, although much work still remains to be done to find a orally effective Fe chelator, a firm foundation of knowledge has been established that will hopefully aid in the development of a clinically useful ligand in the near future.

ACKNOWLEDGMENTS

Work in the authors' laboratories was supported by grants from the Medical Research Council of Canada (P.P., D.R.R.), a Terry Fox New Investigator Award from the National Cancer Institute of Canada (D.R.R.), and the National Health and Medical Research Council of Australia (grant 97060; D.R.R.). D.R.R. was supported by a Fellowship from the Department of Medicine, University of Queensland.

REFERENCES

1. Olivieri NF: Long-term therapy with deferiprone. Acta Haematol 95: 37, 1996.
2. Diav-Citrin O, Koren G: Oral iron chelation with deferiprone. Pediatr Clin North Am 44:235, 1997.
3. Olivieri NF, Brittenham GM: Iron chelation therapy and the treatment of thalassemia. Blood 89:739, 1997.
4. Hershko C: Control of disease by selective iron depletion: A novel therapeutic strategy utilizing iron chelators. Bailliére's Clin Haematol 7:965, 1994.
5. Blatt J, Stitely S: Antineuroblastoma activity of desferrioxamine in human cell lines. Cancer Res 47:1749, 1987.
6. Becton DL, Bryles P. Deferoxamine inhibition of human neuroblastoma viability and proliferation. Cancer Res 48:7189, 1988.
7. Donfrancesco A, Deb G, De Sio L, Cozza R, Castellano A: Role of desferrioxamine in tumor therapy. Acta Haematol 95:66, 1996.
8. Richardson DR: Iron chelators as effective anti-proliferative agents. Can J Physiol Pharmacol 75:1164, 1997.
9. Richardson DR: Analogues of pyridoxal isonicotinoyl hydrazone (PIH) as potential iron chelators for the treatment of neoplasia. Leuk Lymphoma 1998 (in press).
10. Yinnon AM, Theanacho EN, Grady RW, Spira DT, Hershko C: Antimalarial effect of HBED and other phenolic and catecholic iron chelators. Blood 74:2166, 1989.
11. Tsafack A, Loyevsky M, Ponka P, Cabantchik ZI: Mode of action of iron(III) chelators as anti-malarials. IV. Potentiation of desferal action by benzoyl and isonicotinoyl hydrazone derivatives. J Lab Clin Med 127:574, 1996.
12. Golenser J, Domb A, Teomin D, Tsafack A, Nisim O, Ponka P, Eling

W, Cabantchik ZI. The treatment of animal models of malaria with iron chelators by use of a novel polymeric device for slow drug release. J Pharmacol Exp Ther 281:1127, 1997.
13. Schulman HM, Hermes-Lima M, Wang EM, Ponka P: In vitro antioxidant properties of the iron chelator pyridoxal isonicotinoyl hydrazone and some of its analogues. Redox Rep 1:373, 1996.
14. Bhattacharya M, Ponka P, Hardy P, Hanna N, Varma DR, Lachapelle P, Chemtob S: Prevention of postasphyxia electroretinal dysfunction with a pyridoxal hydrazone. Free Radical Biol Med 22:11, 1997.
15. Gehlbach P, Purple RL: Enhancement of retinal recovery by conjugated deferoxamine after ischaemia-reperfusion. Invest Ophthalol Visual Sci 35:669, 1994.
16. Karwatowska-Prokopczuk E, Czarnowska E, Prokopczuk A: Combined therapy with dimethylthiourea, diltiazem and amiloride/dimethylamiloride in the ischemic/reperfused heart. Cardiovasc Res 30:70, 1995.
17. Bel A, Martinod E, Menasche P: Cardioprotective effect of desferrioxamine. Acta Haematol 95:63, 1996.
18. Magro AM, Brai M: Evidence for lipoxygenase activity in induction of histamine release from rat peritoneal mast cells by chelated iron. Immunology 49:1, 1983.
19. Shalit M, Tedeschi A, Miadonna A, Levi-Schaffer F: Desferal (desferrioxamine): A novel activator of connective tissue-type mast cells. J Allergy Clin Immunol 88:854, 1991.
20. Lombardo T, Ferro G, Frontini V, Percolla S: High dose intravenous desferrioxamine (DFO) delivery in four thalassemic patients allergic to subcutaneous DFO administration. Am J Hematol 51:90, 1996.
21. Baker E: Biologic screens for iron chelators. Birth Defects 23:49, 1988.
22. Kontoghiorghes GJ: The design of orally active iron chelators for the treatment of thalassemia. Ph.D Thesis, University of Essex, Colchester, U.K., British Library microfilm no. D66194/86, 1982.
23. Olivieri NF, Brittenham GM, Matsui D, Berkovitch M, Blendis LM, Cameron RG, McClelland RA, Liu PP, Templeton DM, Koren G: Iron chelation therapy with oral deferiprone in patients with thalassemia major. N Engl J Med 332:918, 1995.
24. al Refaie FN, Wonke B, Hoffbrand AV: Deferiprone-associated myelotoxicity. Eur J Haematol 53:298, 1994.
25. al Refaie FN, Hershko C, Hoffbrand AV, Kosaryan M, Olivieri NF, Tondury P, Wonke B: Results of long-term deferiprone (L1) therapy: A report by the international study group on oral iron chelators. Br J Haematol 91:224, 1995.
26. Olivieri NF for the Toronto Iron Chelation Group: Randomized trial of deferiprone (L1) and deferoxamine (DFO) in thalassemia major. Blood 88(Suppl 1):651a, 1996 (abstr).
27. Olivieri NF for the Toronto Iron Chelation Group: Long-term follow-up of body iron in patients with thalassemia major during therapy with the orally active iron chelator deferiprone (L1). Blood 88(Suppl 1): 310a, 1996 (abstr).
28. Castriota-Scanderberg A, Sacco M: Agranulocytosis, arthritis and systemic vasculitis in a patient receiving the oral iron chelator L1 (deferiprone). Br J Hematol 96:254, 1997.
29. Dogherty P, Einarson T, Koren G, Sher G: The effectiveness of deferiprone in thalassemia. Blood 90:894, 1997.
30. Chaberek S, Martell AE: Organic Sequestering Agents. New York: John Wiley and Sons, Inc., 1969.
31. Neilands JB: Microbial iron transport compounds (siderophores) as chelating agents. In Martell AE, Anderson WF, Badman DG (eds): Development of Iron Chelators for Clinical Use. New York: Elsevier/North-Holland, 1981.
32. Modell B, Berdoukas V: The Clinical Approach to Thalassemia. New York: Grune and Stratton, 1984.
33. Keberle H: The biochemistry of desferrioxamine and its relation to iron metabolism. Ann NY Acad Sci 119:758, 1964.
34. Yordanova E, Perfanov K, Slivkova L: The influence of desferrioxamine B on the activity of some iron containing enzymes in vitro. Folia Hematol 94:350, 1970.
35. Kattamis C, Fitsialos J, Sinopoulou C: Oral desferrioxamine in young patients with thalassemia. Lancet i:51, 1981.
36. Summers MR, Jacobs A, Tudway D, Perera P, Ricketts C: Studies of desferrioxamine and ferrioxamine metabolism in normal and iron-loaded subjects. Br J Haematol 42:547, 1979.
37. Propper RD, Shurin SB, Nathan DG: Reassessment of the use of desferrioxamine B in iron overload. N Engl J Med 294:1421, 1976.
38. Propper RD, Cooper B, Rufo RR, Nienhuis AW, Anderson WF, Bunn HF, Rosenthal A, Nathan DG: Continuous subcutaneous administration of desferrioxamine in patients with iron overload. N Engl J Med 297:418, 1977.
39. Modell B: Advances in the use of iron chelating agents for the treatment of iron overload. In Brown EB (ed): Progress in Hematology, Vol. XI. New York: Grune & Stratton Inc., 1979, p 267.
40. Brown EB: Candidate chelating drugs: Where do we stand? In Martell AE, Anderson WF, Badman DG (eds): Development of Iron Chelators for Clinical Use. New York: Elsevier/North-Holland, 1981.
41. Pippard MJ, Callender ST, Weatherall DJ: Intensive iron chelation therapy with desferrioxamine in iron-loading anaemias. Clin Sci Mol Med 54:99, 1978.
42. Grady RW, Peterson CM, Jones RL, Graziano JH, Bhargava KK, Berdoukas VA, Kokkini G, Loukopoulos D, Cerami A: Rhodotorulic acid-investigation of its potential as an iron chelating drug. J Pharmacol Exp Ther 209:342, 1979.
43. Baker E, Wong A, Peter H, Jacobs A: Desferrithiocin is an effective iron chelator in vivo and in vitro but ferrithiocin is toxic. Br J Haematol 81:424, 1992.
44. Hoyes KP, Porter JB: Subcellular distribution of desferrioxamine and hydroxypyridin-4-one chelators in K562 cells affects chelation of intracellular iron pools. Br J Haematol 85:393, 1993.
45. al Refaie FN, Sheppard LN, Nortey P, Wonke B, Hoffbrand AV: Pharmacokinetics of the oral iron chelator deferiprone (L1) in patients with iron overload. Br J Haematol 89:403, 1995.
46. Kontoghiorghes GJ, Evans RW: Site specificity of iron removal from transferrin by α-ketohydroxypyridone chelators. Fed Eur Biochem Soc Lett 189:141, 1985.
47. al Refaie FN, De-Silva CE, Wonke B, Hoffbrand AV: Changes in transferrin saturation after treatment with the oral iron chelator deferiprone in patients with iron overload. J Clin Pathol 48:110, 1995.
48. Cooper CE, Lynagh GR, Hoyes KP, Hider RC, Cammack R, Porter JB: The relationship of intracellular iron chelation to the inhibition and regeneration of human ribonucleotide reductase. J Biol Chem 271: 20291, 1996.
49. Kontoghiorghes GJ. Iron mobilisation from ferritin using α-oxohydroxy heteroaromatic chelators. Biochem J 233:299, 1986.
50. Harrison PM, Arosio P: The ferritins: Molecular properties, iron storage function and cellular regulation. Biochim Biophys Acta 1275:161, 1996.
51. Berkovitch M, Laxer RM, Inman R, Koren G, Pritzker KP, Fritzler MJ, Olivieri NF: Arthropathy in thalassemic patients receiving deferiprone. Lancet 343:1471, 1994.
52. Collins AF, Fassos FF, Stobie S, Lewis N, Shaw D, Fry M, Templeton DM, McClelland RA, Koren G, Olivieri NF: Iron balance and dose-response studies of the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one (L1) in iron-loaded patients with sickle cell disease. Blood 83:2329, 1994.
53. Kersten MJ, Lange R, Smeets ME, Vreugdenhil G, Roozendaal KJ, Lameijer W, Goudsmit R: Long-term treatment of transfusional iron overload with the oral iron chelator deferiprone (L1): A Dutch multicenter trial. Ann Hematol 73:247, 1996.
54. Ponka P, Borova J, Neuwirt J, Fuchs O: Mobilization of iron from reticulocytes. Identification of pyridoxal isonicotinoyl hydrazone as a new iron chelating agent. Fed Eur Biochem Soc Lett 97:317, 1979.
55. Ponka P, Borova J, Neuwirt J, Fuchs O, Necas E: A study of intra-

cellular iron metabolism using pyridoxal isonicotinoyl hydrazone and other synthetic chelating agents. Biochim Biophys Acta 586:278, 1979.
56. Hoy T, Humphreys J, Jacobs A, Williams A, Ponka P: Effective iron chelation following oral administration of an isoniazid pyridoxal hydrazone. Br J Haematol 43:443, 1979.
57. Cikrt M, Ponka P, Necas E, Neuwirt J: Biliary iron excretion in rats following pyridoxal isonicotinoyl hydrazone. Br J Haematol 45:275, 1980.
58. Hershko C, Avramovici-Grisaru S, Link G, Gelfand L, Sarel S: Mechanisms of in vivo chelation by pyridoxal isonicotinoyl hydrazone and other imino derivatives of pyridoxal. J Lab Clin Med 98:99, 1981.
59. Kim BK, Huebers HA, Finch CA: Effectiveness of oral iron chelators assayed in the rat. Am J Hematol 24:277, 1987.
60. Richardson DR, Hefter GT, May PM, Webb J, Baker E: Iron chelators of the pyridoxal isonicotinoyl hydrazone class III. Formation constants with calcium(II), magnesium(II), and zinc(II). Biol Metals 2:161, 1989.
61. Vitolo LMW, Hefter GT, Clare BW, Webb J: Iron chelators of the pyridoxal isonicotinoyl hydrazone class Part 2. Formation constants with iron(III) and iron(II). Inorg Chim Acta 170:171, 1990.
62. Richardson DR, Wis Vitolo ML, Hefter GT, May PM, Clare BW, Webb J, Wiliarat P: Iron chelators of the pyridoxal isonicotinoyl hydrazone class part 1. Ionisation characteristics of the ligands and their relevance to biological properties. Inorg Chim Acta 170:165, 1990.
63. Huang AR, Ponka P: A study of the mechanism of action of pyridoxal isonicotinoyl hydrazone at the cellular level using reticulocytes loaded with non-heme ^{59}Fe. Biochim Biophys Acta 757:306, 1983.
64. Richardson DR: Mobilization of iron from neoplastic cells by some iron chelators is an energy-dependent process. Biochim Biophys Acta 1320:45, 1997.
65. Baker E, Richardson DR, Gross S, Ponka P: Evaluation of the iron chelation potential of pyridoxal, salicylaldehyde and 2-hydroxy-1-naphthylaldehyde using the hepatocyte in culture. Hepatology 15:492, 1992.
66. Richardson DR, Ponka P: The iron metabolism of the human neuroblastoma cell. Lack of relationship between the efficacy of iron chelation and the inhibition of DNA synthesis. J Lab Clin Med 124:660, 1994.
67. Richardson DR, Tran E, Ponka P: The potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective antiproliferative agents. Blood 86:4295, 1995.
68. Richardson DR, Milnes K: The potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective anti-proliferative agents, II: The mechanism of action of ligands derived from salicylaldehyde benzoyl hydrazone and 2-hydroxy-1-naphthylaldehyde benzoyl hydrazone. Blood 89:3025, 1997.
69. Brittenham GM: Pyridoxal isonicotinoyl hydrazone: An effective chelator after oral administration. Semin Hematol 27:112, 1990.
70. Richardson DR, Ponka P: Pyridoxal isonicotinoyl hydrazone and its analogues: Potential orally effective iron-chelating agents for the treatment of iron overload disease. J Lab Clin Med 131:306, 1998.
71. Grady RW, Jacobs A: The screening of potential iron chelating drugs. In: In Martell AE, Anderson WF, Badman DG (eds): Development of Iron Chelators for Clinical Use. New York: Elsevier/North-Holland, 1981, pp 133–164.
72. Pitt CG: Structure and activity relationships of iron chelating drugs. In Martell AE, Anderson WF, Badman DG (eds): Development of Iron Chelators for Clinical Use. New York: Elsevier/North-Holland, 1981, pp 105–131.
73. Hershko C, Grady RW, Link G: Phenolic ethylenediamine derivatives: A study of orally effective iron chelators. J Lab Clin Med 103:337, 1984.
74. Grady RW, Salbe AD, Hilgartner MW, Giardina PJ: Results from a phase I clinical trial of HBED. Adv Exp Med Biol 356:351, 1994.
75. Grady RW, Salbe AD, Hilgartner MW, Giardina PJ: Oral iron chelation: Further development of HBED. Blood 86(Suppl 1):484a, 1995 (abstr).
76. Thorstensen K: Hepatocytes and reticulocytes have different mechanisms for the uptake of iron from transferrin. J Biol Chem 263:16837, 1988.
77. Page MA, Baker E, Morgan EH: Transferrin and iron uptake by rat hepatocytes in culture. Am J Physiol 246:G26, 1984.
78. Trinder D, Morgan EH, Baker E: The mechanisms of iron uptake by rat fetal hepatocytes. Hepatology 6:852, 1986.
79. Richardson DR, Baker E: Two saturable mechanisms of iron uptake from transferrin in human melanoma cells: The effect of transferrin concentration, chelators and metabolic probes on transferrin and iron uptake. J Cell Physiol 161:160, 1994.
80. Richardson DR, Baker E: The uptake of iron and transferrin by the human melanoma cell. Biochim Biophys Acta 1053:1, 1990.
81. Breuer W, Epsztejn S, Millgram P, Cabantchik ZI: Transport of iron and other transition metals into cells as revealed by a fluorescent probe. Am J Physiol 268:1354, 1995.
82. Breuer W, Epsztejn S, Cabantchik ZI: Iron acquired from transferrin by K562 cells is delivered into a cytoplasmic pool of chelatable iron(II). J Biol Chem 270:24207, 1995.
83. Morgan EH: Membrane transport of non-transferrin-bound iron by reticulocytes. Biochim Biophys Acta 943:428, 1988.
84. Richardson DR, Ponka P: The molecular mechanisms of the metabolism and transport of iron in normal and neoplastic cells. Biochim Biophys Acta 1331:1, 1997.
85. Ponka P, Beaumont C, Richardson DR: Function and regulation of transferrin and ferritin. Semin Hematol 35:1, 1998.
86. St. Pierre T, Richardson DR, Baker E, Webb J: A low spin iron complex in human melanoma and rat hepatoma cells and a high spin iron(II) complex in rat hepatoma cells. Biochim Biophys Acta 1135: 154, 1992.
87. Thorstensen K, Aisen P: Release of iron from diferric transferrin in the presence of rat liver membranes: No evidence of a diferric transferrin reductase. Biochim Biophys Acta 1052:29, 1990.
88. Sun IL, Navas P, Crane FL, Morre DJ, Low H: NADH diferric transferrin reductase in liver plasma NADH diferric transferrin reductase in liver plasma membrane. J Biol Chem 262:15915, 1987.
89. Thorstensen K, Romslo I: Uptake of iron from transferrin by isolated rat hepatocytes. A redox-mediated plasma membrane process? J Biol Chem 263:8844, 1988.
90. Porter JB, Singh S, Epemolu RO, Ackerman R, Huehns ER, Hider RC: Oral efficacy and metabolism of 1,2-diethyl-3-hydroxy-pyridin-4-one in thalassemia major. Blood 78(Suppl 1):207a, 1991 (abstr).